Revised Draft

The Evolution of Diet During the 5th to 2nd millennium BC for the population buried at Tepe Hissar, North-eastern Central Iranian Plateau: The Stable Isotope Evidence

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Abstract
This study investigated subsistence economy and dietary changes during the Chalcolithic and Bronze Ages (the 5th to 2nd millennium BC) in the Central Iranian Plateau through a study of skeletal remains buried at Tepe Hissar, Iran. Tepe Hissar experienced widespread socio-cultural and economic transitions during this period. These changes were accompanied by conflict, site abandonment, and reoccupation. This research hypothesised that these socio-cultural and economic changes impacted the subsistence economy and diet of the population. $\delta^{13}C$ and $\delta^{15}N$ was analysed in human bone collagen from 69 adult male and female skeletons from Chalcolithic and Bronze Age Tepe Hissar (Hissar I, II, and III Period). The data showed no significant change in diet during this time, with both sexes from different age-categories having a similar diet. This data did not support the working hypothesis stating that some dietary changes, probably, had occurred in this long period. The isotopic evidence suggested a mixed diet based on C_3 terrestrial plants, animal protein, and a small proportion of fresh water resources. Thus the Tepe Hissar population may have had access to similar food resources during the three millennia of its existence, possibly due to climate continuity in this region. However, the remarkable cultural changes evidenced at this site appear not to have had a significant impact on the diet of people during this time.

1. Introduction and background to the study

The site of Tepe Hissar, located in the north-east region of the Central Iranian Plateau (Fig. 1), has evidence to suggest that it underwent several socio-cultural and economic changes during its existence (late 5th to the early 2nd millennium BC). These are evidenced as changes in pottery style and use of metals, a differentiation in mortuary practices, site abandonment and reoccupation, and large changes in the frequency of interpersonal violence (Schmidt, 1937; Afshar et al 2018). As part of a wider project to advance understanding of population movement and replacement, and the impact of sociocultural and economic changes on mobility, subsistence economy, diet, health, and levels of interpersonal violence during the Chalcolithic and Bronze Ages of Iran (Afshar, 2015), this paper uses carbon and nitrogen stable isotope analysis of human remains to test the hypothesis that these socio-cultural and economic changes impacted the diet of the population. The materials available for analysis were limited to adults only. This paper therefore aims to:

(i) Investigate the impact of socio-cultural and economic transitions and population changes on the subsistence economy and diet of the inhabitants buried at this site, and
(ii) Explore whether there were any differences in diet between males and females and between different adult age categories during the three periods represented at the site.

1.1. The archaeological sequence at Tepe Hissar

Tepe Hissar (Fig.1 and 2) is a complex of disconnected irregular series of mounds and flat areas with a total area of about 12 ha (Dyson and Tosi, 1989). The archaeological sequence indicated a sudden appearance and expansion of the settlement in the late 5th millennium BC, denoted the Hissar I period (4300-3700 BC) (Schmidt, 1937; Majidzadeh, 2008:69, 74). The archaeological evidence from the earliest settlement showed an elaborate cultural assemblage indicating considerable wealth and craft specialization (Pigott et al., 1982). There has been no archaeobotanical or zooarchaeological study to date of Hissar I, but archaeological evidence such as mortars and mullers (for crushing and grinding cereal grains) discovered from this period suggest an agriculturally based society where crops of wheat (*triticum*) or barley (*Hordeum vulgare*) were grown and people may have consumed a mixed diet based on farmed food, including domesticated animals (e.g., sheep (*Ovis aries*), cattle (*Bos taurus*)), alongside wild resources such as gazelle, ibex (*Capra ibex*), mouflon (*Ovis orientalis*-subspecies of wild sheep) and birds (Schmidt, 1937:298). Some of the painted animal figurines, dated to Hissar I have decoration mostly of shapes of sheep (*Ovis aries*), dogs (*Canis lupus familiaris*), goats (*Capra aegagrus hircus*), and cattle (*Bos Taurus*) (Schmidt, 1933). These animal figurines suggest that these species were exploited for their working capacity in agriculture or for food.

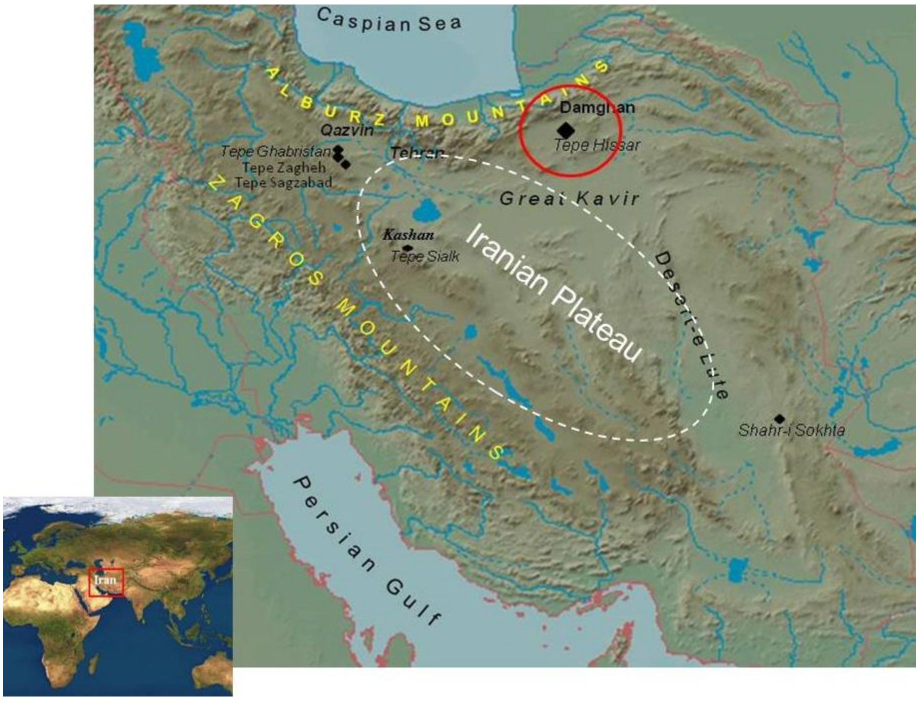

Fig 1. Map of Iran and geographic location of Tepe Hissar (redrawn from Wikimedia: https://commons.wikimedia.org/wiki/File:Whole_world_-_land_and_oceans.jpg based on NASA - Visible Earth and Wikimedia, https://commons.wikimedia.org/wiki/File:Persian_Plateau.png, credit: Dbachmann CC-BY-SA licence)

During the 4th millennium B.C., the site underwent an extreme cultural shift and entered a new era, or the Hissar II period (ca. 3700-2900 BC) (Schmidt, 1933, 1937, Afshar, 2015). A transition from "painted" pottery to "classic grey pottery", combined with changes in architectural style, burial practice, a remarkable increase in industrial activities, the development of craft specialization, and long distance trade, have all been explained by the "arrival of the Hissar II people" into the site (Schmidt, 1937; Dyson, 1987; Dyson and Remsen, 1989). Indeed, cranial and dental metrical and non-metric analyses showed the presence of new people in the Hissar II period (Afshar, 2015). The archaeological evidence of fire and destruction of buildings (Schmidt, 1937; Dyson and Remsen, 1989) as well as bioarchaeological evidence for of violent trauma during this period indicates that these cultural changes were accompanied by conflict and violence (Afshar, 2015; Afshar et al., 2018). The presence of grinding stones in each house indicates that these "Hissar II people" were familiar with cultivating cereals such as wheat and prepared them for cooking (Schmidt, 1937:121; Dyson and Remsen, 1989). Such tools could be used for other types of domestic work.

Nevertheless, in the very early 3rd millennium BC (early Bronze Age, c. 2900 BC) a new phase began, named Hissar III (2900-1700 BC), perhaps due to a endogenous force or foreign influence (Schmidt, 1937:306). Polished grey pottery predominated in this period and was different from the grey pottery found from Hissar II. These changes were accompanied by intensive craft specialization, and social differentiation during the period (Schmidt, 1933, 1937; Tosi, 1989). The presence of mullers and mortars again suggest that people practiced agriculture and food preparation similarly to previous periods (Schmidt, 1937).

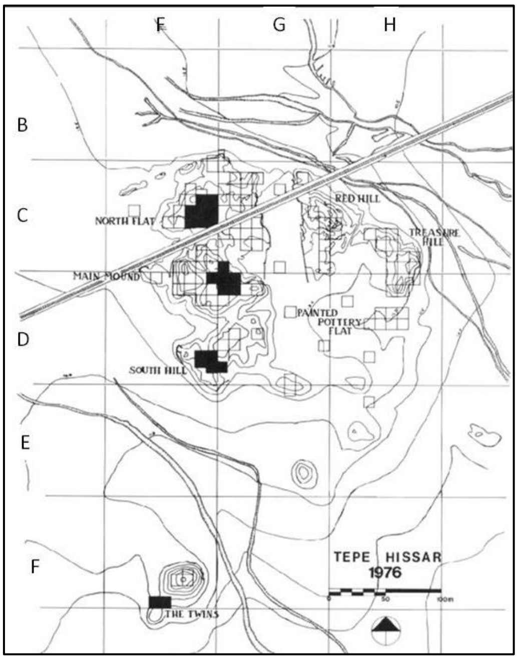

Fig 2. Plan of Tepe Hissar excavations; the black squares represent the area excavated by the re-study team in 1976, and the white squares are those areas excavated by Schmidt in 1931-33 (Dyson and Tosi, 1989)

1.2. Environmental context

Placing the site in environmental context, the settlement of Tepe Hissar was established in the south-eastern slopes of the Alburz Mountains and in a semi-arid/arid zone on the northeast part of Iranian Plateau. The mean annual temperature range in this region is about 14.4 °C (in June-July) to the lowest -17 °C in December-January (Meder, 1989:7-8). The site is also south-east of the Caspian Sea, which lies on the northern side of the Alburz mountains. In the north, limited to the slopes of the Alburz Mountains, there are juniper forest and some trees produce fruits/nuts (Bobek, 1968:287). However, in the adjacent Central Plateau, the amount of vegetation decreases and the landscape turns to steppe and even true desert at lower elevations throughout the plateau (Bobek, 1968:287-8). The Damghan Plain and Tepe Hissar lie at the edge of a desert lake basin and at the foot of alluvial fans that emanate from the Alburz Mountains into the Kavir-e-Damghan (a salt lake: Meder, 1989:8-9). Based on geomorphological and ecological evidence from Tepe Hissar, Meder (1989:11) hypothesised that from 18,000 to around 4500 BC (around the beginning of the Hissar I phase of the settlement) the Kavir-e-Damghan was larger compared to today, was of low salinity, and contained fresh water. Since then and up to the present it has had a tendency toward high salinity. Studies at Tepe Hissar show that the location of this site was ideal for settlement and early agriculture during the Chalcolithic and Bronze Age periods (Dyson and Tosi, 1989; Meder, 1989).

The site was first excavated in the 1930s by Erich Schmidt (Schmidt, 1933, 1937), and in 1979 a re-investigation project was undertaken by the University of Pennsylvania Museum, Philadelphia, USA, Turin University (Italy), and the Iran Centre for Archaeological Research, Tehran (Dyson and Howard, 1989). In more recent times (1995, 2006 and 2010), research was carried out solely by an Iranian team, directed by Yaghmaei and Roustaei (Roustaei, 2006, 2010).

2. Diet at Tepe Hissar: archaeobotanical, archaeozoological and bioarchaeolgical evidence

Archaeobotanical study at Tepe Hissar has demonstrated that most plants cultivated and consumed during mid Hissar II to late Hissar III (3400-1700 BC) belonged to various species of wheat (*Triticum monococcum, T. dicoccum, T. aestivum s.I., Triticum sp*.) and barley (*Hordeum distichum, H. vulgare var. nudum* - Costantini and Dyson, 1990), with little evidence of legumes (e.g., peas, lentils). There is also evidence of fruits (*Vitis* and *Olea*) that are typical of Mediterranean agriculture (Costantini and Dyson, 1990). However, there is no archaeobotanical report from Hissar I. During Hissar III (2900- 1700 BC) an important role in the subsistence economy was also played by mammals of which 73% were domestic (e.g., cattle, sheep, pig, goat), and 27% wild (e.g., gazelle, red deer), together with birds (e.g., *Alectoris chukar*, a gamebird of the pheasant family, Mashkour and Yaghmayi, 1998).

Freshwater fish were also consumed during Hissar III (214 fish bones found, e.g., the freshwater Cyprinidae family) and molluscs (Mashkour and Yaghmayi, 1998; Radu et al., 2008). Goats and cattle were the most common domestic animals in Hissar III. Unfortunately, while the only available archaeozoological report for the Hissar I and II periods is limited it does indicate evidence for freshwater fish being accessed (e.g., Cyprinidae) during these periods (Tosi and Bulgarelli, 1989:45-47; Meder, 1989), thus confirming continuity of access throughout the three periods. The mollusc (Lymnocardiidae/cockle) and freshwater fish bones are the same as species that can be found in the Caspian Sea, suggesting that there may have been some exchange with the population on the other side of the Alburz Mountains at that time, particularly during Hissar III (Mashkour and Yaghmayi, 1998; Radu et al., 2008).

Bioarchaeological analysis of dental caries has shown that caries rates in people who lived during Hissar I (5.5% per tooth) and III (6% per tooth) were consistent with a mixed diet of carbohydrates and animal proteins (Afshar, 2015). However, during Hissar II (2% per tooth), the health of their teeth showed more similarity to pre-agricultural hunter-gatherer populations, who consumed animal protein and low carbohydrate plant foods. Based on worldwide survey of populations from different subsistence groups, Turner (1979) indicates a lower prevalence of caries in hunting and gathering economies (1.7%) compared to mixed economies (4.4%), and agriculturally based populations show the highest rate of caries at 8.6% of teeth affected. These differences between periods, however, were statistically insignificant. The same study indicated that males in the Hissar I period may have had a diet containing a higher carbohydrate content compared to females, who had more access to animal protein in their diet. Sex differences in caries rates declined during Hissar II and III, suggesting males and females had access to similar amounts of carbohydrates. Age categories (all the samples were adult 18+ years) had no significant effect on caries prevalence. The data showed that for all periods, people from the different age-categories experienced caries equally and probably had access to similar amounts of carbohydrates. In addition, this data suggests similarity in subsistence patterns, food preparation techniques, and oral-hygiene for all periods at this site (Larsen, 2018).

Analyses of dental wear showed heavy dental attrition in each period. Almost a quarter of individuals from Hissar I were affected, and during Hissar II and III nearly half of the individuals showed heavy dental attrition (Afshar, 2015). These data suggest that the population at Tepe Hissar may have consumed a "coarse diet" with more grit and fibre in their foods; this could be related to the use of quern stones for making flour from cereal grains, and consumption of various nuts and seeds. In addition, inadequate food preparation time (e.g., uncooked or partly cooked-food), or possibly the consumption of foods which require extensive chewing, such as dried meat and fish, or bone, would have greatly accelerated dental wear. It is assumed that a low prevalence of heavy dental wear during Hissar I indicates the consumption of softer and/or less gritty foods.

Males during Hissar I experienced the lowest rates of advanced dental attrition, but this sharply increased during Hissar II. However, the rate declined among Hissar III males, suggesting that Hissar II and III males consumed more coarse/gritty diets than those who lived during Hissar I. There was no difference between females from the three periods. The difference in dental attrition between the sexes was only significant for Hissar II, when males experienced a higher prevalence of heavy dental wear than females. This suggests that dietary and behavioural variability for both sexes during Hissar II, with males possibly having a different diet or more access to abrasive foods compared to females. It may also indicate possible differences in division of labour or status between males and females in this period, with males possibly using their teeth as tools in occupationally related activities (e.g., making baskets - Afshar, 2015).

3. Isotopes and palaeodiet: background

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios are well established as a tool for dietary reconstruction in archaeology (Richards and Hedges, 1999; Keenleyside et al., 2009).

The major variations in $\delta^{13}C$ derive from differences in fractionation in plant photosynthetic pathways and the use of dissolved bicarbonate rather than carbon dioxide by marine plants. The majority of plants, including trees, wheat and barley, are C_3 photosynthesisers, with tissue $\delta^{13}C$ of -22 to -34‰. C_4 plants are a group of grasses adapted to hot climates in arid and semi-arid regions, including millet and many wild grasses, and having $\delta^{13}C$ of -9 to -16‰ (van der Merwe, 1982). Fewer species, mostly cacti and succulents, follow the Crassulacean acid metabolism, with intermediate $\delta^{13}C$, and they are of little importance in human diets (Lajtha and Marshall, 1994). Marine plants have $\delta^{13}C$ of -18‰ to -16‰ (Sealy et al., 1995, Grupe et al., 2009). Smaller variations in $\delta^{13}C$ of C_3 plants (3-6‰) occur as a result of environmental factors, with high humidity, high altitude, and low temperature causing decreases $\delta^{13}C$, and aridity causing an increase (Tieszen, 1991; Lajtha and Marshall, 1994). The carbon isotopic composition of plants is reflected in the food-chains based on them, with small shifts (about +1‰) with each trophic level and a further fractionation of about +4‰ into bone collagen (DeNiro and Epstein, 1978; Vogel and van der Merwe, 1977).

Nitrogen isotope ratios in organisms vary primarily through an increase of 3-6‰ in $\delta^{15}N$ for each trophic level in a food chain (Bocherens and Drucker, 2003). Nitrogen fixing terrestrial plants, such as legumes, have mean $\delta^{15}N$ of 0 to 4‰, but the majority of terrestrial plants obtain their nitrogen from the soil and have $\delta^{15}N$ of about 3‰ (Peterson and Fry, 1987). There are, however, other factors that can significantly increase $\delta^{15}N$. These include increases potentially as large as a trophic level shift caused by manuring of crops (Bogaard et al., 2007), nutritional stress and starvation in animals (Hobson et al., 1993; McCue and Pollock, 2008; Gaye-Siessegger et al., 2007) and aridity or salinity (Hartmann, 2011, Britton et al., 2008). In marine ecosystems $\delta^{15}N$ is elevated and variable with primary producers at -2 to +10‰ (Cabana and Rasmussen, 1996), and long food-chains leading to $\delta^{15}N$ of up to 20‰ in top predators (Schoeninger et al., 1983).

From the analysis of bone collagen, prehistoric humans can be placed within ecosystems and inferences made about their diets in terms of the extent of carnivory, and the use of aquatic resources and the exploitation of C_4 resources. Collagen isotope ratios primarily, but not wholly, reflect the protein component of diet (Fernandes et al., 2012) and must be interpreted in light of natural variations in $\delta^{13}C$ and $\delta^{15}N$ outlined above. In the absence of archaeozoological samples, however, it is not possible to interpret whether the proteins consumed come from domestic animals or wild terrestrial animals, or egg or dairy and so on. Similarly, it is not easy to interpret if an increase in $\delta^{15}N$ was due to a shift from plant to animal protein, environmental change, manuring or even diseases or nutritional stresses (Afshar 2015).

4. Materials and methods

4.1 Materials

The excavations by Schmidt (1933, 1937) at Tepe Hissar uncovered 1637 human skeletons, of which 397 (24 %, adult and non-adult) are curated at the University of Pennsylvania's Penn Museum, in the Department of Archaeology and Anthropology. Unfortunately, the rest of the skeletons may have been reburied or curated in an unknown place in Iran. It is not known whether Schmidt selected these remains randomly, by sex or age, or based his selection on the presence of disease, the place where he uncovered them, preservation/completeness, or perhaps period or other unknown criteria. The skeletal remains at Penn Museum are dated from the Chalcolithic to the Bronze Age (late 5th -2nd millennium B.C- Hissar I, II and III), from an "unknown" period, and the Islamic period (Middle Islamic Period ~1400 AD). The focus of this research was the human remains dating from the early Chalcolithic to the Bronze Age (late 5th- 2nd millennium B.C.). From the 368 adult individuals available for study from these periods, bone samples from 69 individuals were selected for isotopic analysis to represent both males and females from all three periods (Hissar I, II, III; Table. 1). Unfortunately, no faunal or botanical remains were available for analysis.

4.2 Methods

4.2.1 Determination of sex and age

Multiple ageing and sex estimation methods were utilized. Estimation of sex was based on sexually dimorphic traits of the cranium and mandible (Acsádi and Nemeskéri, 1970:87-90; Buikstra and Ubelaker, 1994:19-20; Loth and Henneberg, 1996) and pelvis (Phenice, 1969; Acsádi and Nemeskéri, 1970:75-79; Buikstra and Ubelaker, 1994:16-19; Bass, 1995:202). Measurements of long bones such as the femoral, humeral and radial-head diameters, the femoral-bicondylar width, clavicle length, and scapula-glenoid cavity width were also recorded to aid sex estimation (Bass, 1995; Afshar, 2015). Skeletons with ambiguous traits were assigned indeterminate (unknown sex).

Age-at-death estimation was based on the final stages of growth including molar eruption (van Beek, 1983; Ubelaker, 2004:64), and fusion of the spheno-occipital synchondrosis, the iliac crest, the ischial tuberosity, the first two segments of the sacrum, and the sternal end of the clavicle (Black and Scheuer, 1996; Scheuer and Black, 2000:4-17). Morphological and degenerative changes also examined included cranial suture closure (Meindl and Lovejoy, 1985), degenerative changes in the auricular surface of the ilium (Lovejoy et al., 1985a), pubic symphysis morphology (Brooks and Suchey, 1990), and dental attrition (Miles, 1962, 1963; Brothwell, 1981:72). Other age related traits that are more likely present in older adults were also considered, including antemortem tooth loss and osteoporosis (Lovejoy et al., 1985b), and joint disease (osteoarthritis: Rogers and Waldron, 1995). The age categories utilized were based on Buikstra and Ubelaker's (1994:36) recommendations, but to obtain more nuanced information, the young adult class was divided into two: young adult 1 (18-25 years), young adult 2 (26-35 years), middle adult (36-50 years), old adult (50+), and adult (18+) (Afshar, 2015).

4.2.2. Sample collection and preparation

Samples from the mid shaft cortex of long bones were obtained from the Penn Museum under the direction of Dr. Janet Monge, and the samples were processed at Durham University.

Collagen extraction followed a modified Longin procedure (1971; Brown et al., 1988) as described by Smits et al. (2010). A subsample of 90 to 200 mg was taken from each sample, and demineralized in 0.5M HCl at 4˚C for several days. The demineralized samples were washed with purified water, filtered, gelatinized at pH 3.0 for 24 to 48 hours at 75ºC and ultra-filtered with the >30kDa fraction retained. After lyophilization samples were weighed and yields calculated. Samples with less than 1% yield were rejected (van Klinken, 1999). Each sample was measured in duplicate. Between 0.30 and 0.35 mg of purified freeze-dried gelatin was weighed into tin capsules. Total carbon and nitrogen content, and stable isotope analysis of the collagen samples were performed using a Costech Elemental Analyzer (ECS 4010) connected to a Thermo Delta V Advantage isotope ratio mass spectrometer. Carbon isotope ratios were corrected for ^{17}O contribution and reported relative to Vienna Pee Dee Belemnite (VPDB). Nitrogen isotope ratios are reported against atmospheric N_2 (AIR). Isotopic accuracy was monitored through routine analyses of international standards and in-house standards. Analytical uncertainty was calculated using replicate analyses, typically ±0.1‰ for analyses of the international standards and typically <0.2‰ on sample analyses. Samples were considered unreliable and discarded if they had C:N ratios outside 2.9 to 3.6 (DeNiro, 1985) or elemental concentrations outside 35-50 % (C%) or 11-16 % (N%) (van Klinken, 1999).

4.2.3. Statistical analysis

Statistical analyses were performed using SPSS, Version 20. Differences in $\delta^{13}C$ and $\delta^{15}N$ between males and females in each period were tested using a Mann-Whitney test, and between age groups using a Kruskal-Wallis test. Differences in $\delta^{13}C$ and $\delta^{15}N$ between the three periods by pooled sex were tested using the Kruskal-Wallis test and Levene's test. The significance level was set at 0.05.

5. Results

5.1. Collagen preservation

The results of the isotopic measurements and basic descriptions of the individuals analysed are displayed in Table. 1. The bone samples were very well preserved. Of 69 samples analysed, 68 yielded collagen of sufficient quality, with only one sample from Hissar III rejected due to a yield of less than 1 wt %.

Table. 1. Samples and isotopic results for carbon and nitrogen at Tepe Hissar

Sample no.*	**Square**	**Sk no.**	**Sex**[1]	**Age**[2] (year)	**Bone**	**Collagen yield** (wt.%)	**$\delta^{13}C$** $_{VPDB}$ (‰)	**$\delta^{15}N$** $_{AIR}$ (‰)	**C:N**	**C** (wt.%)	**N** (wt.%)
Hissar I (n=8)											
A7	CG95	16	M	YA1	Humerus	10.9	-19.5	11.1	3.3	44.1	15.6
A23	DH21	12	M	YA2	Femur	10.4	-19.2	12.0	3.2	42.7	15.4
A18	DG69	16	M	AA	Tibia	12.7	-19.9	10.9	3.3	44.0	15.4
A17	DG69	8	F	YA1	Femur	9.4	-20.0	11.3	3.2	42.0	15.6
A9	DG36	2	F	AA	Femur	11.1	-19.9	12.1	3.2	42.3	15.3
A5	CG95	8	F	AA	Tibia	11.4	-19.7	12.5	3.2	42.5	15.5
A2	CG95	4	F	YA1	Tibia	8.6	-19.7	11.8	3.3	44.1	15.8
A20	DG96	8	I	AA	Humerus	11.4	-20.4	13.1	3.3	41.8	14.8
Hissar II (n=11)											
A36	CG25	20	M	AA	Humerus	7.4	-20.1	11.4	3.1	42.0	15.6
A35	CG25	13	M	AA	Femur	11.8	-19.2	11.7	3.2	42.2	15.6
A34	CG25	5	M	AA	Humerus	8.8	-20.1	13.5	3.2	42.0	15.3
A32	CG25	1	M	MA	Femur	13.3	-18.9	12.5	3.2	41.9	15.4
A121	DF29	5	M	MA	Radius	9.1	-19.2	13.4	3.2	44.0	15.9
A128	DF29	28	F	YA1	Femur	10.1	-19.8	13.1	3.2	41.2	15.1
A38	CG25	23	F	AA	Femur	15	-19.8	10.8	3.2	42.1	15.2
A29	DG96	1	F	AA	Femur	13.4	-19.1	12.7	3.2	42.8	15.7
A39	CG60	4	F	YA1	Humerus	6.9	-20.2	12.7	3.3	42.4	15.2
A42	DG96	22	F	YA2	Femur	13.4	-19.3	10.6	3.2	42.5	15.6
A33	CG25	4	I	AA	Tibia	11.4	-19.4	11.8	3.3	42.0	14.9
Hissar III (n=49)											
A101	DF19	29	M	AA	Femur	8.6	-19.1	11.8	3.4	42.6	14.9
A143	DG10	7	M	MA	Femur	13.6	-18.9	13.0	3.1	41.9	15.6
A66	DF18	9	M	YA2	Femur	13.3	-19.8	12.6	3.1	41.9	15.6
B158	CH86	4	M	OA	Femur	14.3	-20.4	11.8	3.3	41.7	14.8
A70	DF18	15	M	YA1	Femur	13.9	-20.4	14.1	3.5	43.8	14.8
A79	DF18	38	M	YA2	Femur	9.5	-19.6	11.9	3.2	41.7	15.3
B102	DG11	16	M	YA2	Femur	11	-19.0	13.1	3.2	42.3	15.4
B110	DG20	18	M	YA2	Femur	7.3	-20.1	12.9	3.2	41.8	15.4
B111	DG20	21	M	MA	Femur	11	-19.8	12.3	3.2	41.7	15.2
B76	CG90	4	M	YA2	Femur	11	-19.9	11.9	3.2	42.4	15.4
B80	CG90	23	M	AA	Femur	11	-18.6	13.3	3.3	44.7	15.6
A98	DF19	23	M	AA	Tibia	7.3	-19.5	13.9	3.3	43.3	15.4
A71	DF18	16	M	MA	Femur	11.6	-19.8	11.8	3.3	43.8	15.7
A133	DG00	1	M	MA	Femur	10.3	-19.3	12.5	3.2	43.7	15.8
A60	DF09	1	M	AA	Femur	13.4	-18.4	12.6	3.3	44.1	15.5

B120	CG90	1	M	MA	Femur	15	-19.8	10.7	3.3	44.1	15.6
A117	DF29	1b	M	YA2	Femur	13.7	-19.8	11.8	3.1	41.8	15.5
B103	DG11	32	M	AA	Femur	14.4	-20.2	12.4	3.4	44.1	15.0
A124	DF29	8	M	MA	Femur	14	-19.8	13.4	3.3	44.1	15.4
A135	DG00	4	M	AA	Femur	10.2	-19.8	12.3	3.3	44.5	15.8
A181	DF18	17	M	AA	Femur	12.2	-19.2	12.5	3.3	44.1	15.5
A118	DF29	2	M	YA2	Humerus	14.7	-20.0	11.7	3.1	42.3	15.7
B116	CF79	1	M	AA	Femur	15	-19.7	12.5	3.4	43.6	15.2
A45	EG06	5	M	AA	Tibia	11.6	-19.9	11.2	3.2	42.4	15.7
A205	DG00	8	F	YA2	Femur	16.8	-20.2	12.1	3.2	41.5	15.3
A141	DG00	22	F	MA	Femur	12.5	-19.5	11.1	3.2	41.8	15.5
A182	DF18	18	F	MA	Femur	11.5	-20.3	12.6	3.2	41.4	15.1
A206	DG00	8	F	YA2	Femur	14.9	-19.9	12.5	3.2	41.8	15.2
A81	DF18	39a	F	MA	Femur	13.2	-20.0	11.5	3.2	42.1	15.2
A94	DF19	17	F	AA	Femur	11.2	-19.8	13.1	3.2	42.0	15.4
A95	DF19	19	F	MA	Femur	13.6	-19.8	12.2	3.2	42.6	15.3
B185	DG01	15	F	MA	Tibia	13.8	-19.3	13.0	3.3	41.7	14.9
B226	DG20	17	F	YA1	Femur	12	-20.1	11.0	3.2	41.9	15.2
A110	DF19	55	F	YA2	Femur	12.7	-19.7	11.8	3.1	41.4	15.5
A99	DF19	24	F	MA	Humerus	11.4	-18.7	12.8	3.2	41.9	15.2
A136	DG00	5	F	YA2	Femur	9.2	-19.9	12.6	3.2	42.9	15.5
A87	DF19	4	F	AA	Femur	12.9	-19.8	13.1	3.2	41.8	15.3
A97	DF19	21	F	MA	Femur	11	-18.1	12.8	3.2	42.3	15.6
B101	DG01	38	F	AA	Femur	7.3	-19.7	12.3	3.1	41.8	15.5
B58	CF55	1	F	AA	Femur	7.3	-19.6	12.4	3.2	42.1	15.4
B79	CG90	15	F	YA2	Humerus	7.3	-20.0	11.9	3.3	42.9	15.1
A89	DF19	7	F	YA1	Femur	13.5	-19.9	11.4	3.3	44.5	15.7
A167	DG00	19	F	AA	Tibia	13.8	-19.0	8.8	3.2	43.6	15.8
B119	CG80	2	F	YA1	Femur	14.1	-19.5	11.9	3.2	43.1	15.6
B122	CH64	2	F	AA	Femur	13.6	-19.7	10.6	3.3	44.2	15.9
B106	DG11	52	F	YA1	Femur	15	-20.2	12.0	3.4	43.4	15.1
B178	DG01	1	F	AA	Femur	11	-20.0	13.2	3.3	43.9	15.3
A204	DG00	7	I	AA	Tibia	11.8	-19.9	11.9	3.1	42.4	15.8
A47	EG06	29	F	YA2	Femur	9.9	-19.5	11.8	3.6	45.7	15.0
A142 (Failed)	DG10	3	F	YA2	Femur	0.7	-	-	-	-	-

[1]M=Male, F=Female, and I=Indeterminate
[2]YA1=18-25 years, YA2=26-35 years, MA=36-50 years, OA=50+ years, AA=18+years
*A= Museum no 33-16-sk. no., B= Museum no 33-23-sk. no. (e.g. 33-16-20, 33-23-185)

Table 2. Summary statistics

Period		Males			Females			Sexes compared		Age groups compared	
		Mean	Min	Max	Mean	Min	Max	U	p	K-W	p
I	$\delta^{13}C$	-19.5	-19.9	-19.2	-19.8	-20.0	-19.6	3	0.289	3.2	0.201
	$\delta^{15}N$	11.3	10.8	12.0	11.9	11.3	12.5	2	0.157	1.8	0.400
II	$\delta^{13}C$	-19.5	-20.1	-18.9	-19.6	-20.1	-19.1	10	0.602	5.2	0.152
	$\delta^{15}N$	12.5	11.4	13.5	12.0	10.6	13.1	9	0.465	4.4	0.223
III	$\delta^{13}C$	-19.6	-20.4	-18.4	-19.7	-20.2	-18.1	249	0.565	8.0	0.092
	$\delta^{15}N$	12.4	10.7	14.1	12.0	8.7	13.2	229	0.317	1.3	0.865

5.2. Carbon and nitrogen stable isotope values by period

Hissar I: There was little variation in both $\delta^{13}C$ and $\delta^{15}N$ among individuals (Table 2, Fig. 3). However, the small sample size from this period should be considered. Comparison between the sexes, showed males having slightly more positive $\delta^{13}C$ (0.3‰) than females, in contrast, females displayed slightly higher $\delta^{15}N$ values (0.6‰) compared to males. These differences between males and females were not significant. There was thus an insignificant difference in diet between individuals from different age groups.

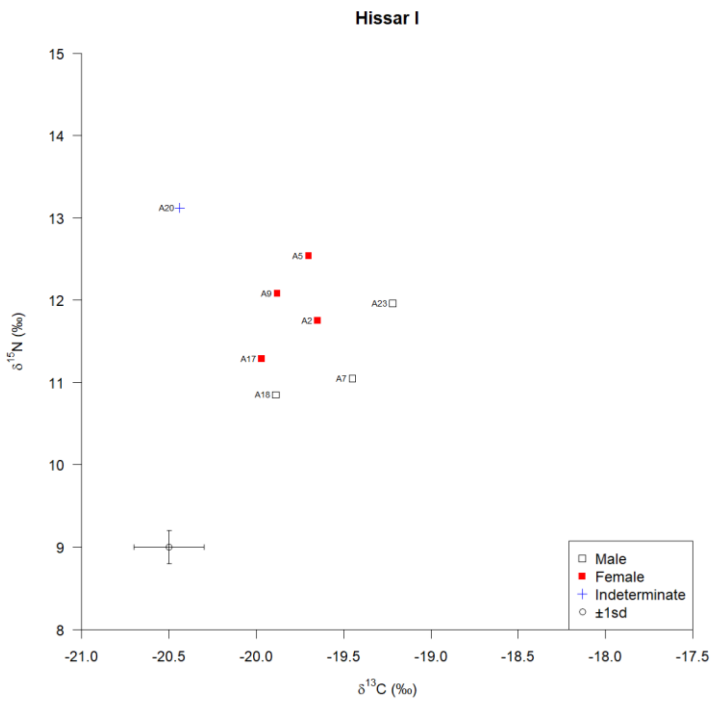

Fig 3. Carbon and nitrogen stable isotope ratios of bone collagen from Hissar I, by sex

Hissar II: Figure 4 shows $\delta^{13}C$ versus $\delta^{15}N$ for the eleven individuals from this period. There was little sex difference in diet in people during Hissar II (Table 2, Fig. 4). Males had marginally higher $\delta^{13}C$ (0.1‰) and $\delta^{15}N$ (0.5‰) compared to females. $\delta^{15}N$ showed a marginally wider range among females compared to males. These differences between the sexes were not significant. The data showed a small difference in carbon and nitrogen values between different age groups.

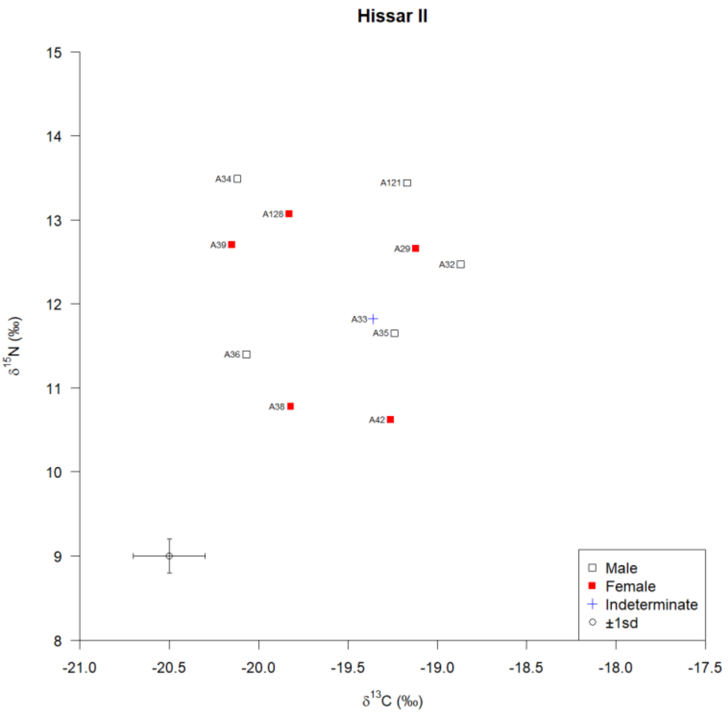

Fig 4. Carbon and nitrogen stable isotope ratios of bone collagen from Hissar II, by sex

Hissar III: Figure 5 shows a plot of δ^{13}C versus δ^{15}N for 49 individuals from Hissar III. The data showed little sex or age difference in diet in Hissar III (Table 2, Fig 5). The mean values for δ^{13}C and δ^{15}N were marginally higher in males (by 0.1‰ and 0.4‰ respectively) compared to females. However, these differences between the sexes for δ^{13}C and δ^{15}N values and between age groups during Hissar III were not significant. One female (A167) was an outlier with a lower δ^{15}N value (8.8‰).

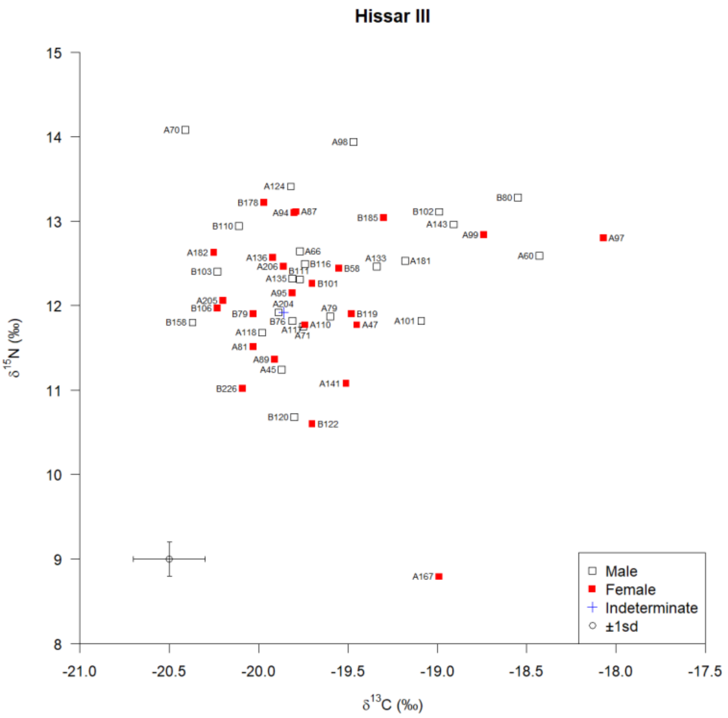

Fig 5. Carbon and nitrogen stable isotope ratios of bone collagen from Hissar III, by sex

5.3. Carbon and nitrogen stable isotope ratios: between periods by pooled sex and age

A comparison of $\delta^{13}C$ versus $\delta^{15}N$ values between periods and by pooled sex at Tepe Hissar is illustrated in Figure 6 and Table 3. In general, the SD for $\delta^{13}C$ values was smaller for Hissar I individuals compared to Hissar II and Hissar III. The SD for $\delta^{15}N$ values was smaller during Hissar I compared to Hissar II and Hissar III.

Table. 3 Comparison of the isotopic values between periods at Tepe Hissar (pooled sex and age)

Period	No.	Mean $\delta^{13}C$‰	SD	Range‰	Mean $\delta^{15}N$‰	SD	Range‰
Hissar I	8	-19.8	0.4	-20.4 to -19.2	11.8	0.8	10.8-13.1
Hissar II	11	-19.5	0.5	-20.1 to -18.9	12.2	1.0	10.6-13.5
Hissar III	49	-19.6	0.5	-20.4 to -18.1	12.2	0.9	8.8-14.1
All	68	-19.6	0.5	-20.4 to -18.1	12.2	0.9	8.8-14.1
		Kruskal-Wallis	**Levene**		**Kruskal-Wallis**	**Levene**	
Test statistic		0.644	0.589		1.558	0.567	
p		0.718	0.558		0.459	0.570	

Figure 6 shows that, with passing time at Tepe Hissar, $\delta^{13}C$ and $\delta^{15}N$ values were shifted slightly in a positive direction, particularly among Hissar III individuals. Overall, the data shows that, during Hissar II both $\delta^{13}C$ and $\delta^{15}N$ ratios increased slightly (0.2‰ and 0.4‰, respectively) compared to Hissar I, but the mean isotopic signatures for Hissar III stayed almost identical to Hissar II. However, these differences in both $\delta^{13}C$ and $\delta^{15}N$ values among individuals from the three periods were not significant. A Levene's test also did not show any significant differences in variance.

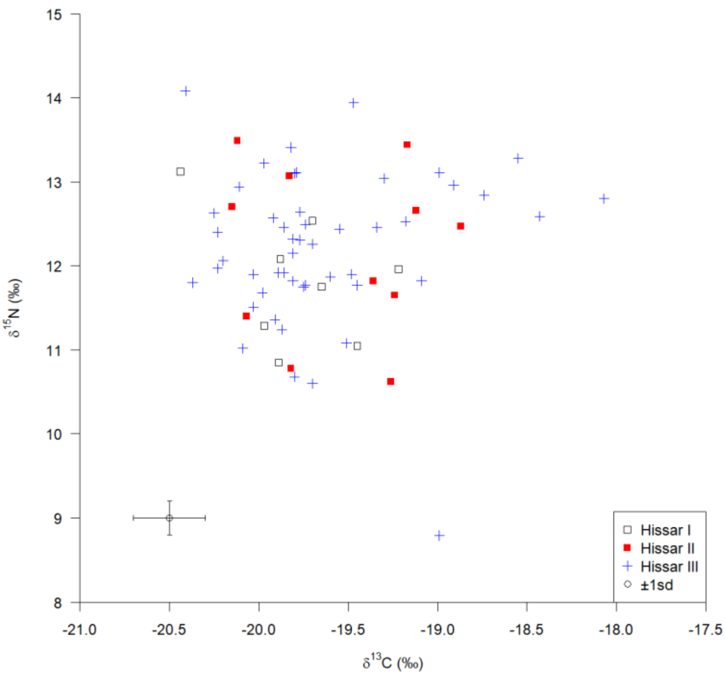

Fig 6. A comparison of carbon and nitrogen stable isotope ratios of bone collagen at Tepe Hissar by pooled sex and age

6. Discussion

It was hypothesised that socio-cultural and economic transitions and events that occurred at Tepe Hissar during the 5th to the 2nd millennium BC, and particularly during Hissar II and III. This went alongside population influxes, which together impacted the subsistence economy and diet of people within and between periods; this also differed between males and females and different age groups. However, the mean carbon and nitrogen isotope ratios from Tepe Hissar pointed to similar isotopic compositions, indicating isotopically similar diets for all three periods at this site, and providing no evidence to support the hypothesis. Males, females and different age groups in each period also did not show significant isotopic differences in diet.

Although our data indicate the possible isotopic composition of human diet, they do not represent the food class, quality or proportions of foods consumed (Hedges et al., 2008). The interpretation of diet in ancient populations must also consider that the isotopic composition of humans can be influenced by non-dietary factors such as environmental changes (e.g., in aridity or the land or salinity of expanses of water), biological variability, physiological factors (e.g., starvation, pregnancy, etc.), and bone remodelling rates (Ambrose, 1991; Hobson et al., 1993; Fuller et al., 2004, 2005; Hedges and Reynard, 2007). As we see no changes, it seems unlikely that there were major shifts in these factors. However, there may have been changes in the species of both plants and animals consumed without changes in isotope composition, for example replacing emmer with barley..

The mean $\delta^{13}C$ at Tepe Hissar did not change significantly when compared between periods, and is consistent with a C_3 terrestrial diet for all periods. This is supported by archaeobotanical evidence from Hissar II and III, showing that most plants, including cereals such as wheat and barley, fruits, and vegetables cultivated and consumed at Tepe Hissar belonged to the C_3 pathway (van der Merwe and Vogel, 1983; Costantini and Dyson, 1990). The majority of individuals at Tepe Hissar (Figure 6) have a $\delta^{13}C$ between -20.4‰ and -19.0‰ (C_3 pathway), but four individuals (2 male and 2 female) from Hissar III have $\delta^{13}C$ between -18.7‰ and -18.1‰, suggesting that they may have had access to a different diet that was not common at Tepe Hissar at that time. Higher $\delta^{13}C$ values probably indicate a small proportion of C_4 terrestrial foods in their diet (either plants or terrestrial animals that consumed them), and/or possibly a small amount of marine food. It is possible that these individuals may have come to the site from another region with a different ecosystem and/or food resources, or that the resource was brought to Tepe Hissar. Males showed slightly less negative $\delta^{13}C$ than females in each period, but these differences were insignificant. This finding suggests females had access to similar food resources as males during each period. In contrast, dental caries data from Hissar I indicated males may have had more carbohydrate in their diet compared to females.

The $\delta^{15}N$ for Tepe Hissar showed insignificant difference between periods (Table 3), suggesting a similar consumption of animal protein for all periods. The mean $\delta^{15}N$ for each period indicates consumption of a mixed diet with a significant amount of animal protein (e.g., meat or dairy produce). This result is consistent with the dental caries study that indicated a mixed diet for all periods. This also corresponds to zooarchaeological data from Hissar III, demonstrating the presence of different domestic and wild mammal species, birds, and freshwater fish and molluscs at the site (Meder, 1989; Mashkour and Yaghmayi, 1998; Radu et al., 2008). For Hissar I and II the limited zooarchaeological data and animal figurines would appear to indicate a similar mixture. Furthermore, the faunal remains from Hissar III indicate the importance of animal stock breeding at the site (e.g., goats); goats and cattle were the most common domestic animals during Hissar III; cattle were kept for traction and killed when they were older (Mashkour and Yaghmayi, 1998).

The $\delta^{15}N$ of humans is elevated relative to foods consumed by 3-5‰ on average, whether they are local plants, herbivore/carnivores species, or aquatic resources (DeNiro and Epstein, 1981). However, local conditions such as soil salinity or arid environments (Ambrose, 1991; Tieszen, 1991; Hedges and Reynard, 2007; Hartmann 2011) can increase the $\delta^{15}N$ in plants and animals living in those areas, consequently increasing $\delta^{15}N$ in other trophic levels and the whole foodweb.

Lying in an arid or semi-arid region such as the Damghan plain, Tepe Hissar is likely to show a large range of $\delta^{15}N$ values in plants and animals. Unfortunately, there is no available information regarding the $\delta^{15}N$ or even $\delta^{13}C$ (modern or ancient) for botanical or faunal species from Tepe Hissar to predict the diet of this population. There were also no animal bone samples available for isotope analysis. Therefore, we used isotopic values from the sites of Tepe Zagheh (ZAG), Qabrestan (QAB), and Sagzabad (SAGZ) (dates 4960-863 B.C) located on the Qazvin Plain, which is another arid/semi-arid region in the Central Iranian Plateau. The $\delta^{15}N$ for domestic (8.0‰ to 12.0‰, mean=10‰) and wild (6.9‰ to 10.2‰, mean=8.5‰) herbivores, and dogs (11.6‰ to 14.5‰, mean=13‰) were considered as a base for the Tepe Hissar human isotopic data (Bocherens et al., 2000; Figure 7). It was expected that individuals with a purely vegetarian diet at Tepe Hissar would exhibit $\delta^{15}N$ similar to domesticated herbivores from the Qazvin plain (mean=10‰). However, the $\delta^{13}C$ for the terrestrial domesticated animals from the Qazvin plain were higher (-16.6‰ to -19.0‰, mean= -17.8‰ - Bocherens et al., 2000) than for the individuals from Tepe Hissar. Therefore, it could be that the environment and climate at Tepe Hissar during the 5th to the 2nd millennium BC was less arid or less saline compared to the Qazvin region, or pastures used at Qazvin may have had a higher C_4 grass component. Furthermore, the high $\delta^{15}N$ for this site may have had no link to arid conditions, as for the Qazvin plain (Bocherens et al., 2000), since the majority of individuals exhibited a $\delta^{13}C$ lower than -19.0‰. It seems that other factors, for example higher proportions of terrestrial animal protein and/or freshwater fish in the diet, may have been responsible for a high $\delta^{15}N$ at Tepe Hissar (see below).

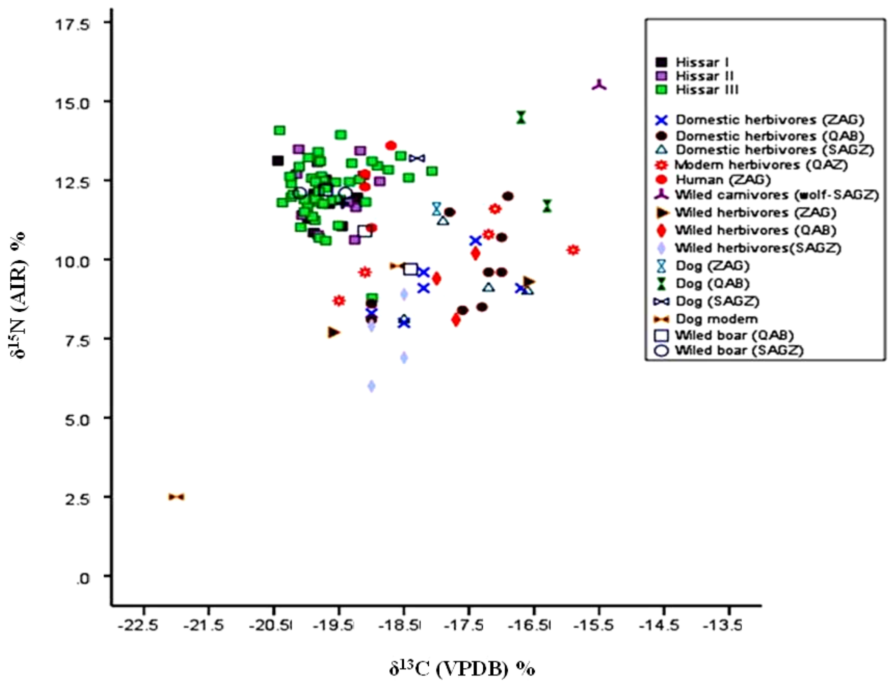

Fig 7. Comparison of isotopic data between Tepe Hissar (this study) and the Qazvin Plain (Bocherens et al., 2000)

The range of distribution of $\delta^{15}N$ was slightly narrower for Hissar I compared to Hissar II and III, but was the same for both sexes (1.2‰), suggesting similar, limited variability in access to animal and fish protein foods by males as well as by females during Hissar I. These data suggest a diet high in animal protein and/or containing small quantities of freshwater fish, or people at the site perhaps manured the cereal crops that contributed to their diet (Müldner and Richards, 2005; Vika and Theodoropoulou, 2012). The excavations at Tepe Hissar uncovered freshwater resources (fish bones and molluscs) from the Hissar I period (Meder, 1989, Thornton, 2009). None of the individuals from Hissar I showed a purely vegetarian diet or any evidence for C_4 foods.

Three males and three females from Hissar II showed high $\delta^{15}N$ compared to the rest of the individuals from this period (between 12.5‰ and 13.5‰). Their $\delta^{13}C$ was between -20.2‰ and -18.9‰ and consistent with a terrestrial C_3 diet, suggesting consumption of animal protein, and possibly an input to the diet of a small amount of protein from freshwater resources (Müldner and Richards, 2005). The excavation found many fish bones from the Hissar II period (Tosi and Bulgarelli, 1989: e.g., from Cyprinidae fish), supporting consumption of freshwater fish in this period. Two females from this period exhibited a lower $\delta^{15}N$ (10.6‰, 10.8‰, respectively) compared to other females and males; their $\delta^{13}C$ was -19.3‰ and -19.8‰, respectively, suggesting a mixed-diet with lower animal protein and possibly more C_3 plants. None of the individuals from Hissar II showed a purely vegetarian diet.

The overall range of $\delta^{15}N$ for Hissar III was 8.8‰ to 14.1‰, indicating that some individuals had higher $\delta^{15}N$ than might be expected from a terrestrial diet. Therefore, it seems that these individuals consumed a mixed-diet, including local terrestrial animal protein, and probably a small quantity of freshwater resources. As discussed above, three other individuals from this period also showed more positive carbon values. One female (A167) showed a low $\delta^{15}N$ (8.8‰), suggesting this individual possibly had a diet based purely on terrestrial C_3 plants, with a very small/or no animal protein component, or origins in an area where foods had lower $\delta^{15}N$. This individual was discovered from a mass-burial from square DG00 and didn't show any pathological condition or any sign of trauma (Afshar, 2015, Afshar et al., 2018). The rest of the people in this period appear to have had different mixed-diets based on terrestrial C_3 plants and animal protein (perhaps both domestic and wild herbivores) and freshwater resources (Mashkour and Yaghmayi, 1998; Radu et al., 2008).

7. Conclusion

Overall, the carbon and nitrogen stable isotope data showed that the Tepe Hissar population had access to similar food resources across all periods for about 3000 years, from the late 5th to 2nd millennium BC. These data showed that the events that occurred at this site did not significantly impact on the isotopic composition of food resources available and subsequent diet, during each period. Individuals from each period, both females and males from different age-categories, had a similar diet based on C_3 plants and animal protein (a mixed diet), as well as a small contribution from fresh water resources. This finding is consistent with archaeobotanical and zooarchaeological data from Tepe Hissar, suggesting the cereal crops grown were mostly wheat and barley, with supplemental vegetables and fruits, and the animals that contributed to their diet were both wild and domestic. These data also correspond to bioarchaeological studies of dental caries from this site, indicating a mixed diet for the three periods at Tepe Hissar. However, the high percentage of people with heavy dental wear, particularly during Hissar II and III may reflect changes in dietary behaviour, food preparation techniques, or food texture over time to abrasive diet (e.g., raw plant materials, raw meat, dried meat/fish, or bone). Some individuals showed different stable isotope carbon and nitrogen ratios, suggesting the presence of newcomers to the site, as do bioarchaeological studies of cranial and dental metric and non- metric traits from this site (Afshar, 2015). This hypothesis could be tested by further work using strontium and oxygen isotopes, or analyses of ancient DNA.

8. Acknowledgements

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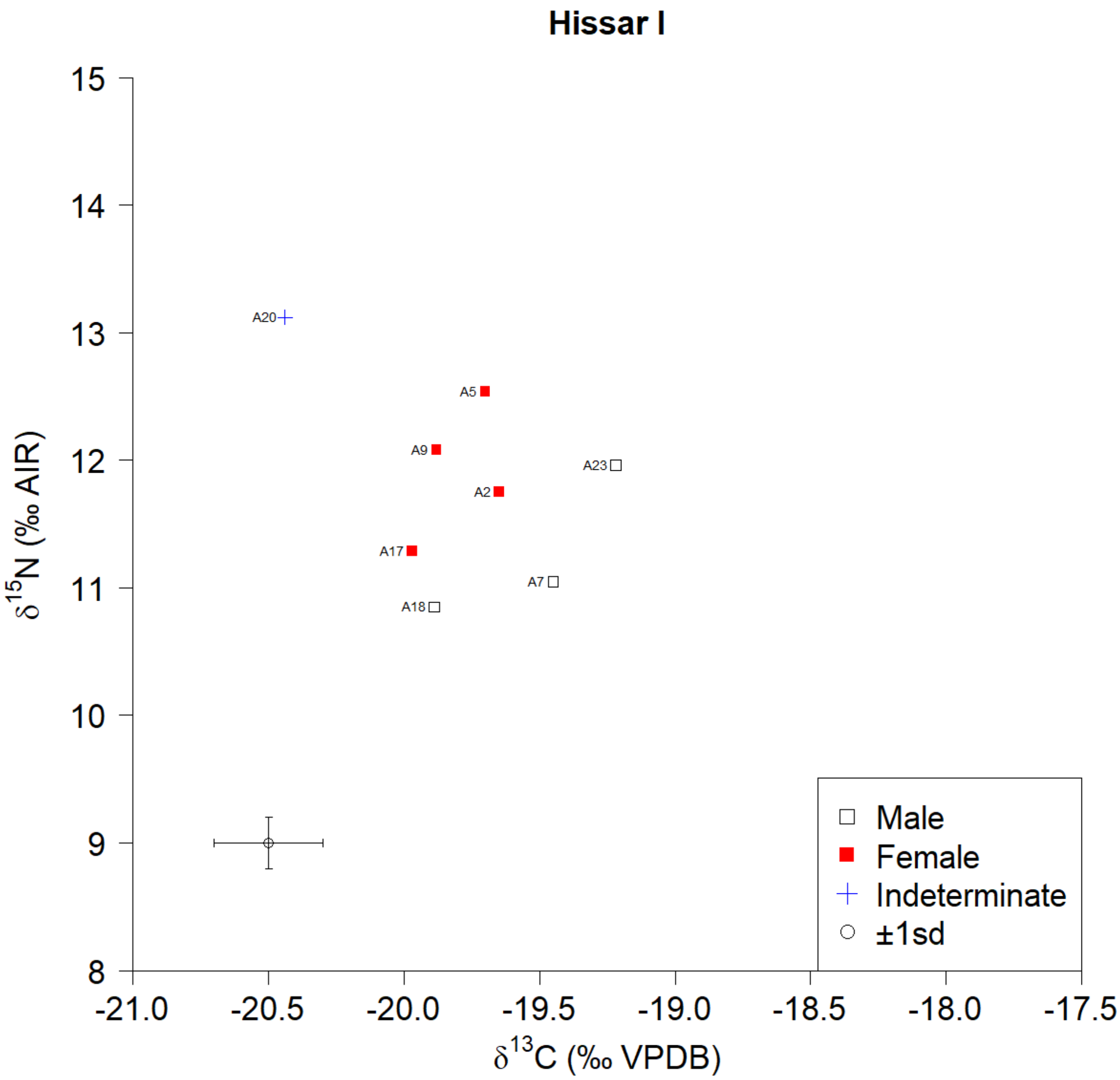

Hissar I
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δ15N (‰ AIR)
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-20.5
-20.0
-19.5
-19.0
-18.5
-18.0
-17.5
δ13C (‰ VPDB)
A20
A5
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A23
A2
A17
A7
A18
Male
Female
Indeterminate
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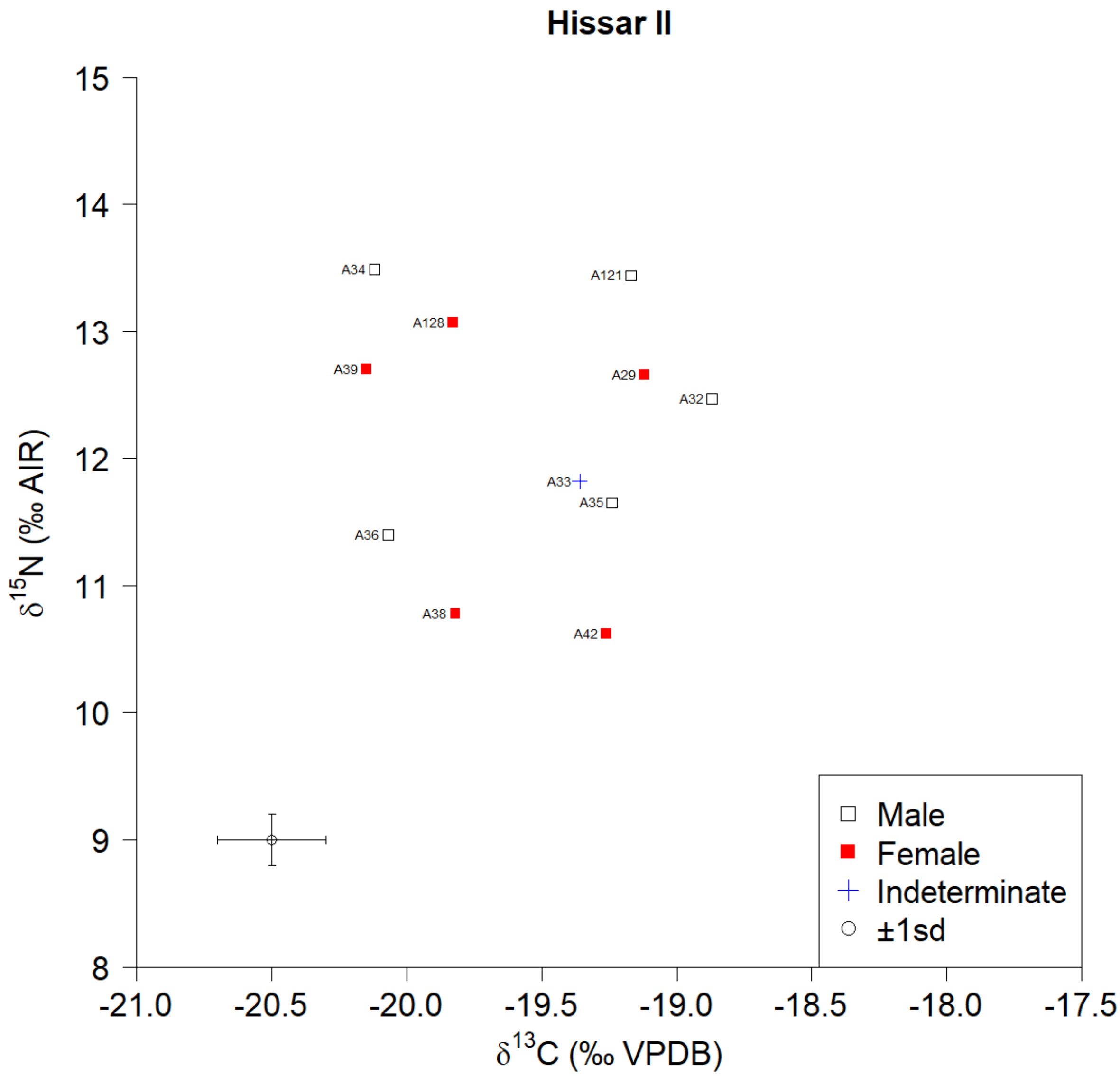

Hissar II
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A39
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A32
A33
A35
A36
A38
A42
Male
Female
Indeterminate
±1sd
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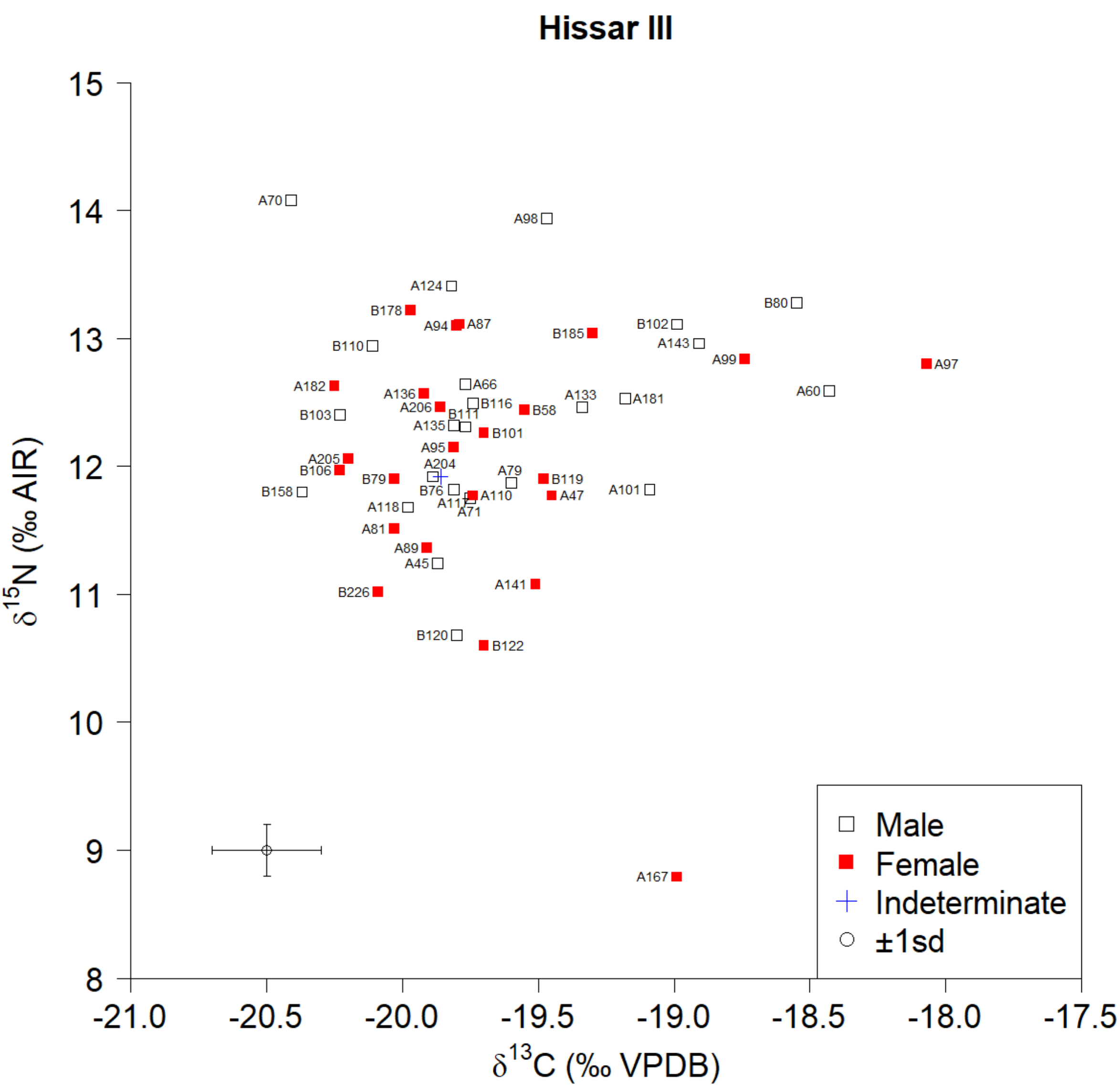
δ13C (‰ VPDB)



Hissar III
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δ13C (‰ VPDB)
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-19.5
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Male
Female
Indeterminate
±1sd
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A98
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B178
A94
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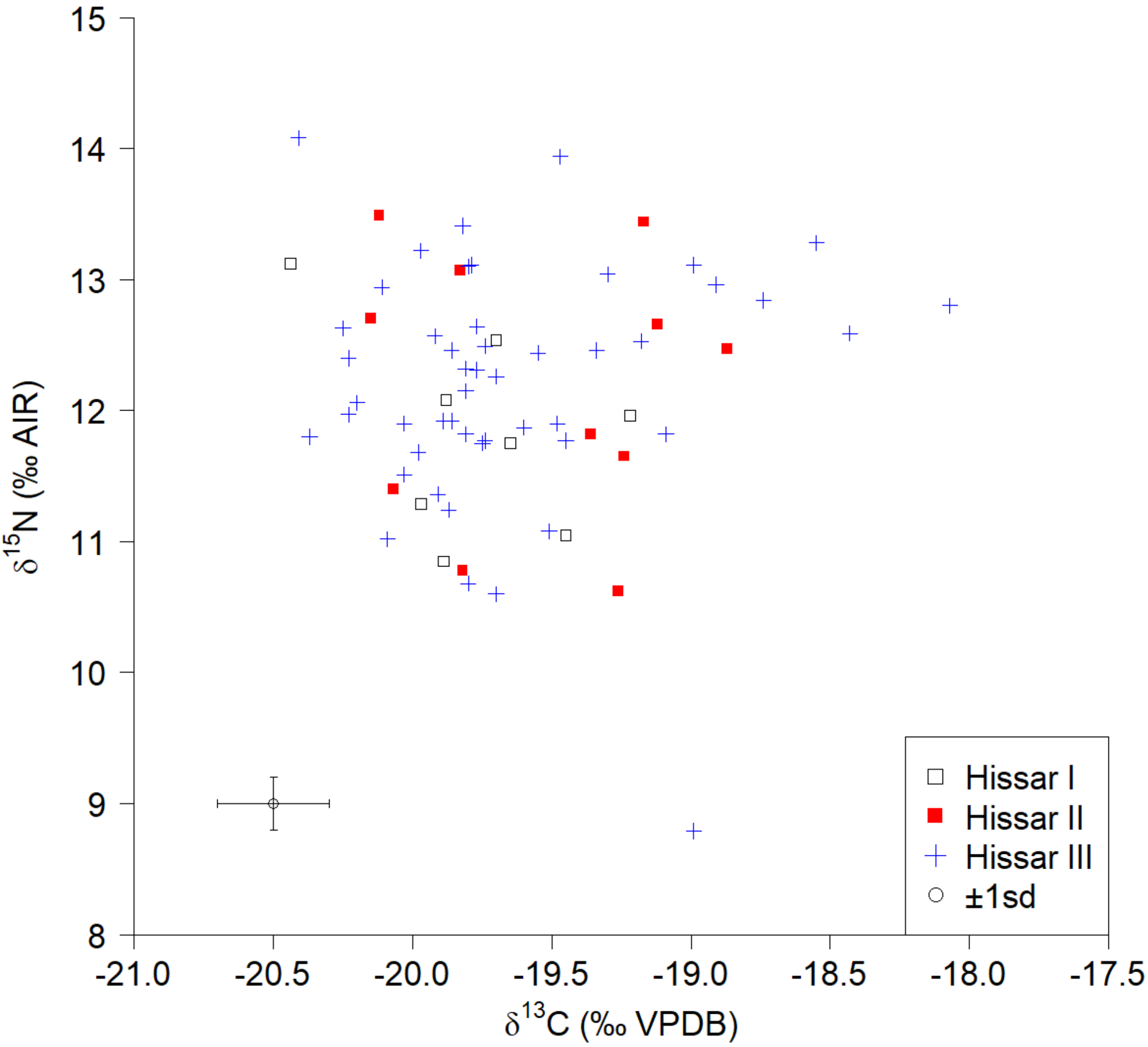
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δ15N (‰ AIR)
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δ13C (‰ VPDB)
Hissar I
Hissar II
Hissar III
±1sd